ABSTRACT

Introduction: Tamarindus indica L or Tamarind as commonly known is a medicinal plant with a broad spectrum of application in medicine. Tamarind is the most useful plant for liver disorders treatment in Cuba and The Caribbean. People use it as decoction and have to take up to four liters a day of this preparation, because the use of alcohol in the Tamarind fluid extracts prevent its use in patient with lever disorders.

Objective: the aim of this study was the preliminary establishment of quality control parameters of the Tamarindus indica L. soft extract as a novel alcohol-free active ingredient for solids pharmaceutical formulation.

Methods: organoleptics properties, total solids, relative density, pH, and total ashes were established. The polyphenols content was evaluated as a chemical marker using Folin-Ciocalteau spectrophotometric method. The WHO methodology was followed for the establishment of limits for each one of the parameters.

Results: the proposed limits were: total solids 63.55-69.22 %; relative density 1.311-1.441g/mL; pH 3.20-3.32; total ashes 1.00-1.21 %; polyphenols content 6.19-8.12 %, and flavonoids 0.35-1.27 %.

Conclusion: the obtained data are a preliminary guide to implement the quality control procedure for further preparation of this novel natural extract, for its utilization as an active ingredient in pharmaceutical formulations.

Key words: soft extract, polyphenols, flavonoids, antioxidant, tamarind, Tamarindus indica.
RESUMEN

Introducción: el *Tamarindus indica* L. o tamarindo, como se le conoce comúnmente, es una planta medicinal con un amplio espectro de aplicación en la medicina. Es la más utilizada en Cuba y en el Caribe para los trastornos hepáticos. La población la utiliza en forma de decocción y consume alrededor de 4 L/d de esta preparación, porque el contenido de alcohol del extracto fluido de tamarindo impide su utilización en pacientes con estas enfermedades.

Objetivo: establecer de forma preliminar los parámetros de control de calidad en el extracto blando de las hojas de *Tamarindus indica* L. como un nuevo ingrediente activo libre de etanol, para la preparación de formas farmacéuticas sólidas.

Métodos: se evaluaron las propiedades organolépticas, sólidos totales, densidad relativa, pH y cenizas totales. Se evaluó el contenido de polifenoles como marcador para el control de calidad de este extracto utilizando el método de Folin Ciocalteau. Se empleó la metodología propuesta por la Organización Mundial de la Salud para el establecimiento de los límites de control.

Resultados: los parámetros propuestos fueron: sólidos totales 63,55-69,22 %; densidad relativa 1,311-1,441 g/mL; pH 3,20-3,32; cenizas totales 1,00-1,21 %; polifenoles 6,19-8,12 % y flavonoides 0,35-1,27 %.

Conclusiones: los datos obtenidos son una guía preliminar para implementar el procedimiento de control de calidad en la preparación de este nuevo extracto natural para su utilización como ingrediente activo en preparaciones farmacéuticas.

Palabras clave: extracto blando, polifenoles, flavonoides, antioxidante, tamarindo, *Tamarindus indica*.

INTRODUCTION

Researches with medicinal plants are focused on the identification of new active molecules; specially, in plants that have a broad ethnopharmacological use. *Tamarindus indica* L. or tamarind as it is commonly known, belongs to the Fabaceae family, Caessalpiniaceae subfamily, and it is a tropical tree, native of Africa, Southern Asia, America and the Caribbean. Tamarind is rich in fatty acids and heavy alcohols. Leaves also contain protein and essential aminoacids, carbohydrates and minerals as zinc, magnesium, phosphorus, copper, selenium, and calcium;\(^1\) volatile oils;\(^2\) steroids, resins, mucilage and sugars are reported in this species too.\(^3\) Tamarind also contains iron, vitamins A, B and C and organic acids like citric, tartaric and malic;\(^4\) as well as polyphenols and flavonoids,\(^4\) who are the main responsible of the strong antioxidant;\(^5,6\) hepatoprotective\(^7,8\) and antimicrobial activity\(^9,10\) of the tamarind leaf extract.

In Cuba, ethnobotanical use of tamarind is extended for the treatment of hepatitis.\(^11\) The ethnopharmacological broad spectrum of *Tamarindus indica* L. leaves extract and all recent studies that confirm its pharmaceutical activity, compelled us to establish the quality control parameters of the tamarind soft extract (TSE) in order to use it as an active ingredient in pharmaceutical formulations.
METHODS

Plant material: tamarind leaves were collected from a tamarind population in El Caney, Santiago de Cuba province. They were previously identified by Dr. Jorge Sierra Calzado. A voucher specimen registered as 052216 was deposited at the teaching section of the BSC herbarium at the Biology Department, Natural Sciences Faculty, Oriente University, Cuba.

Soft extract: soft extract was obtained by vacuum concentration of the fluid extract, in a vacuum evaporation system (KIKA WERKE GMBH & Co. Germany), at temperature of 42 ± 2 °C. Ten one-liter batches every one, were prepared with dried drug collected from September 2009 to June, 2010.

Organoleptics characteristics: organoleptic properties were evaluated by simple inspection through the senses. Color, appearance, smell and taste were evaluated.

Total solids and relative density: these properties were determined according to the British pharmacopoeia.¹²

pH evaluation: the pH measurement was made at 30 °C. One gram of TSE was weighed and diluted in 25mL of distilled water, the solution was filtered. A direct pH value was obtained from a pH-meter (Hanna Instruments, Spain). The pH-meter was calibrated using potassium tetraoxalate 0.05M (pH 1.68 at 30 °C) and potassium dihydrogen citrate 0.05M (pH 3.77 at 30 °C) (both reactive Riedel - de Häiën; Germany) as buffer solutions.¹³

Total ashes: they were determined according to literature¹²,¹³ using a German furnace MLW.

Total polyphenols and flavonoids content: the Folin Ciocalteau¹² analytical procedure was performed and validated to spectrophotometrically measure, polyphenols content in tamarind soft extract whereas flavonoids were determined by spectrophotometrical method reported in the British Pharmacopoeia.¹²

Establishment of limits: Reasonable limits were established through the use of the 3ó statistical method.¹⁴ Ten successive batches were evaluated using 5 samples per batch and the grand average and standard deviation (SD) were computed. Minimum and maximum limits for each parameter were calculated using the following expressions:

Minimum limit= Mean 3*SD (1)

Maximum limit= Mean + 3*SD (2)

Statistical analysis: All assays were made by quintuplicate, and the mean and standard deviation were reported. Statistical analysis was made using Statgraphics Plus 5.0. Stat Ease Co. Minneapolis, USA. An F-test followed by a Tukey HSD test was performed to compare pairs of group mean. An á equal to 0.05 was used. In all cases, the normality of the data were proved to confirm the validity of the statistical analysis.
RESULTS

Organoleptics: tamarind soft extract appears as a dark brownish syrup with strong acidic and ripe fruit flavor, with a viscous and resinous appearance.

Table 1 exhibits the results of the quality parameters evaluated for TSE. Standardized kurtosis and asymmetry for all parameters were between ± 2, indicating the normality of the data (data not reported).

Table 2 shows the proposed parameters for the quality control evaluation of the tamarind soft extract, with the corresponding maximum and minimum limits.

For all evaluated properties in the ten batches, statistical significant differences among mean were founded. Anova test for Total solids showed a F-value of 39.02 and p-value equal to 0.0000; Relative density, F=366.20, p-value = 0.0000; Total ashes, F-value of 366.20 and p-value equal to 0.0000; Ph, F-value of 23.93 and p-value equal to 0.0000; Polyphenols, F-value of 103.09 and p-value equal to 0.0000; Flavonoids, F-value of 245.63 and p-value equal to 0.0000.
DISCUSSION

Organoleptic properties of TSE showed great uniformity in the ten batches studied. The acid taste is linked in literature to the presence of ascorbic, tartaric and citric acids.\textsuperscript{3} The result observed for total solids was caused by variability in the vegetable composition related to different phenologic states of plant.\textsuperscript{15} Relative standard deviation in all batches was below 1\%, this fact highlights the uniformity and validity of the preparation method.

The amount of total solids in TSE is a synonymous of the efficiency in the extractive process, and in a general way, has an influence in the concentration of all compounds present in the extract. For this reason an Anova test was made to evaluate total variability of total solids in the ten batches studied and after that, to propose a standard value. According to this analysis, all parameters in table 2 will show similar values, 95 percent of the time that the tamarind soft extract is prepared by this method.

For relative density, the observed results are coherent with total solid contained in tamarind soft extract, where was observed a statistical relationship between them (data not reported).

For Total ashes it was found that the higher values of TS in the TSE the higher total ashes remains, usually the more efficient extraction process shows the highest amount of total solids and the highest content of ashes.

The pH measures in the ten batches showed the acidity of TSE, peculiar characteristic of this species. As reported in literature, this fact is the result of the presence of organic acids like malic, citric, tartaric, caffeic, ferulic, and ascorbic acids.\textsuperscript{3,4}

Polyphenols and flavonoids are complex groups of substances that usually occur as a mixture and which are difficult to purify and crystallize. They are easily oxidized and polymerized in solution; if this happens they lose much of their astringent effect and are therefore of little therapeutic value.\textsuperscript{16}

Heat affects the bio-pharmaceutical and chemical stability of the polyphenols and flavonoids, therefore this is the most important parameter to consider in the evaluation of the quality parameters of this product because of they are responsibles of the antioxidant activity of this product.

Polyphenols content in batches were significantly different, in the same way, the mean flavonoid content in all batches were significantly different too. This behavior is frequent in natural products, due to natural variability. This is especially true in this species, and variation appears related to climate, collected time, soil characteristics and phenological stages of the plant, being the fructification stage (January to March), the moment where the phenolics and flavonoids compounds in leaves of the plants are higher,\textsuperscript{15} probably due to the need of the plant to protect itself from the insects and other predators attacks.
The organoleptic and physicochemical quality control parameters of tamarind soft extract were established in this paper. The proposed limits were: total solids 63.55-69.22 %; relative density 1.311-1.441 g/mL; pH 3.20-3.32; total ashes 1.00-1.21 %; polyphenols content 6.19-8.12 %, flavonoids 0.27-1.27 %. Obtained data are a preliminary guide to implement the quality control procedure for further preparation of this novel natural extract, for it utilization as an active ingredient in pharmaceutical formulations.

REFERENCES


Recibido: 24 de agosto de 2011.
Aprobado: 15 de noviembre de 2011.

Jesús Rodríguez-Amado. Departament of Pharmacy. Universidad de Oriente. Patricio Lumumba s/n. Santiago de Cuba, Cuba. Teléf.: 53 22 632263. E-mail: jimmy@cnt.uo.edu.cu